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SEED GERMINATION STUDIES

I. PURIFICATION AND PROPERTIES OF AN α-AMYLASE FROM THE COTYLEDONS OF GERMINATING PEAS

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SUMMARY

A starch-hydrolyzing enzyme present in extracts of the cotyledons of germinating peas has been purified over 3400-fold. Several independent criteria show that this activity is due to an amylase of the α-type (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1). The enzyme exhibits maximal activity in the pH range 5.3–5.9; the Michaelis constant (K_m) and energy of activation (E_a) for the enzyme-catalyzed reaction are $2 \cdot 10^{-4}$ g soluble starch per ml and 7600 cal/mole, respectively. Calcium ions protect the amylase against heat inactivation, whereas incubation of the enzyme with $5 \cdot 10^{-3}$ M EDTA for 20 min at room temperature results in complete loss of activity. With amylose and amylopectin as substrates, this enzyme is similar in action pattern to other known plant α-amylases.

INTRODUCTION

The process of seed germination is accompanied by a variety of interesting and significant biochemical phenomena. In a general way, these changes can be described as follows: (1) a breakdown of seed reserves (carbohydrate, fat, and protein); (2) a possible accumulation of resulting intermediates, some of which in themselves are new and novel compounds; and (3) the subsequent utilization of these intermediates in energy-yielding processes or for the synthesis of new plant materials.

More specifically, seed germination is characterized by a changing array of enzymic activities. Some of these activities increase dramatically from an initially low or even undetectable state to an extraordinarily high level; others increase to a moderate level and then decline to an activity lower than initially present; yet another group of enzymic activities are found in the dry seed at an intermediate level which remains constant throughout germination[1–6]. The same type of fluctuating

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changes have been observed for simple metabolites; one of the most striking in this regard is the absence of homoserine in the dry pea seed but the accumulation of this compound as the most predominant free amino acid in the growing seedling after 5–7 days of germination[7–10].

The time-course of many of these events as well as the specific plant tissues in which they occur suggest that they may represent changing metabolic functions related to tissue maturation or differentiation or both. It still remains an open question as to (1) why some enzymic activities change during germination and not others, (2) what the nature of the control processes may be which so specifically govern these changes, as well as (3) what the relationship between those enzymic activities that increase or decrease and the metabolism of specific tissues or of the whole seedling may be. For these reasons, we have initiated studies in this area with the goal of providing new information and correlations on metabolic pathways operative in the germinating seed (and seedling) and on the general mechanisms controlling plant metabolic processes.

In 1959, YOUNG AND VARNER[1] reported that the cotyledons of germinating peas contain amylase activity of an undetermined nature, the activity of which increases manyfold during the process of germination. As a beginning, we examined the characteristics of this amylase and its possible metabolic role in concert with other carbohydrases present in the cotyledon or growing seedling[11]. We attempted, therefore, to prepare the amylase in a high state of purity for the following reasons: (1) it would permit characterization of the enzyme as being of the α- or β-type; (2) the properties of this amylase could then be compared with amylases isolated from other biological sources; and (3) it would enable us to provide a more unequivocal answer to the question of whether the increased enzymic activity is due to synthesis of new protein or to unmasking of the enzyme preformed in seeds. This last point could be tested by isolating the amylase in a high state of purity from cotyledons germinated in the presence of radioactive amino acids.

This paper describes the purification of the pea cotyledon amylase over 3400-fold; it is an amylase of the α-type (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1); values for the Michaelis constant, the activation energy, and the pH optimum of the enzyme have been determined. This amylase requires Ca^{2+} for activity but not Cl^-. Certain features of this work have been reported in abstract form[12].

MATERIALS AND METHODS

Materials

Pea seeds (*Pisum sativum*, early Alaska variety) were purchased from the W. Atlee Burpee Co., Clinton, Iowa (U.S.A.). Commercial samples of an α-amylase and a β-amylase (EC 3.2.1.2), obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio (U.S.A.), were preparations from bacteria and barley malt, respectively. Small samples of maltotriose and maltotetraose for use as chromatographic reference compounds were generously supplied by Dr. R. L. WHISTLER, Purdue University, Lafayette, Indiana (U.S.A.). Dr. I. J. GOLDSTEIN of this department kindly provided us with samples of higher oligosaccharides and cycloheptaamylose. All other chemicals used were commercial products.

Methods

Solutions of soluble potato starch devoid of opalescence, used for optical rotation measurements, were prepared by dissolving 10 g of commercial soluble starch in 200 ml of 1 M HCl. The resulting solution was heated and maintained at boiling temperature for 1 min. The pH value of the warm solution was adjusted to 8.0 by dropwise addition of aq. ammonia (specific gravity, 0.90). This solution was then cooled and dialyzed exhaustively against distilled water. The fluid volume of the solution in the dialysis bag was concentrated 50% at 40° under reduced pressure on a rotary film evaporator. The addition of two volumes of acetone to the resultant solution caused precipitation of the starch .The precipitate was removed by centrifugation and dried in an oven at 60° for 1 h. The final preparation was stored at room temperature over P_2O_5 *in vacuo* in a desiccator. When required, a 1% solution was made by suspending 1 g of the solid in 100 ml of H_2O and bringing the mixture to a boil with constant stirring.

For studying the action pattern of the amylase, amylose solutions were made by refluxing 50 mg of commercial amylose with 5 ml of water for 15 min. Any insoluble material remaining after this treatment was removed by centrifugation. In this manner, approximately a 0.1% solution of amylose is obtained.

Whatman No. 1 filter paper was used together with a solvent system consisting of ethyl acetate–pyridine - water (10:4:3, v/v). Descending solvent flow was used routinely for chromatographic purposes unless otherwise noted. Reducing substances present on chromatograms were detected by the method of TREVELYAN, PROCTOR AND HARRISON[13].

Protein concentrations were determined by the procedure of LOWRY *et al.*[14] with crystalline bovine serum albumin as the standard. In some cases when the protein concentration was very low, as after fractionating protein solutions on columns of calcium phosphate–cellulose, the spectrophotometric method of MURPHY AND KIES[15] was used.

Pea seeds were routinely germinated in the dark on moist, sterile vermiculite at 25°. The dry seeds, as obtained commercially, were first allowed to imbibe water for 4–6 h. They were then washed briefly with a 1% solution of sodium hypochlorite and finally rinsed with water before being placed on vermiculite. Cotyledons from 8–11-day-old plants were used as the source of the enzyme.

The method of BERNFELD[16] was used routinely to assay amylase activity. The substrate for this assay is a 1% solution of soluble starch in 0.1 M acetate buffer (pH 5.6). The enzyme preparation to be tested is diluted to 1 ml with water and then 1.0 ml of the substrate solution is added. After 10 min incubation at 25°, 2 ml of a 3,5-dinitrosalicylic acid reagent are added and the tubes are placed in a boiling water bath for 5 min. The tubes are then cooled to room temperature and the contents of the tubes are finally diluted with 20 ml of water. The absorbance values of the resulting colored solutions are determined in a Klett–Summerson photoelectric colorimeter equipped with a No. 54 filter. A calibration curve established with maltose is used to convert the colorimeter readings into milligrams of maltose. One unit of amylase activity is defined as the quantity of protein that liberates 1 mg of reducing groups (calculated as maltose even though the actual reaction products are dextrins) in 10 min under the conditions described. Specific activity is expressed as units of enzyme activity per mg of protein. Appropriate controls correc-

ted for any endogenous reducing power present in the enzyme preparation being tested.

RESULTS

Purification of the amylase from pea cotyledon extracts

The following operations were carried out between 0° and 4° unless stated otherwise.

Step 1. Cotyledons (150 g) excised from 11-day-old etiolated pea plants and stored at −10° were homogenized with 500 ml of 0.025 M Tris–HCl buffer (pH 7.4) for 1 min in a Waring blendor. The resulting homogenate was strained through cheese cloth and then centrifuged at 18 000 × *g* for 30 min.

Step 2. Calcium chloride solution (20 ml, 0.5 M) was added to the supernatant solution and the mixture was heated with constant stirring for 15 min at 70°. The precipitate formed was removed by centrifugation, and the supernatant fluid strained through glass wool. The resulting clear solution was dialyzed against 0.0125 M Tris–HCl buffer (pH 7.4) overnight. The dialyzed enzyme solution was then lyophilized to dryness yielding 4.1 g of dry powder. In this form, the enzyme preparation is quite stable and can be stored at −10°.

Step 3. Portions (150 mg each) of the lyophilized enzyme powder were added to 8 separate conical centrifuge tubes. The powder was dissolved in 2 ml of 0.025 M Tris–HCl buffer (pH 7.4), and 1.34 ml of cold absolute ethanol were subsequently added to each tube with stirring. The tubes were then allowed to stand at −7° for 1 h with occasional stirring. After centrifuging, the 8 individual supernatant fluids were combined into 4 tubes. To each tube was then added 0.4 ml of 0.025 M Tris–HCl buffer (pH 7.4) followed by 0.2 ml of glycogen reagent[17] and 0.4 ml of cold absolute ethanol. This mixture was stirred for 10 min at −7° and then centrifuged. The glycogen pellet present in each tube was washed twice by suspending in 1.67 ml of a mixture of 40% ethanol and 60% 0.01 M acetate buffer (pH 5.8) − 0.005 M $CaCl_2$. After washing, the pellets were individually dissolved in 1 ml of 0.01 M acetate buffer (pH 5.8) containing 0.005 M $CaCl_2$. The 4 separate solutions containing the enzyme and glycogen were incubated at 37° for 1 h and then combined. Enzyme preparations at this stage of purity were routinely used for studies pertaining to the properties of the amylase.

Step 4. A calcium phosphate-cellulose column, 1 cm × 4.5 cm, was prepared as described by MASSEY[18]. The calcium phosphate–cellulose was initially equilibrated with 0.01 M phosphate buffer (pH 6.5) after which 3.5 ml of the enzyme solution obtained in Step 3 were carefully layered on the top of the column and allowed to soak into the column bed. The cellulose column was first washed with 16 ml of 0.01 M phosphate buffer (pH 6.5). The enzyme was then eluted from the cellulose with 0.06 M phosphate buffer (pH 6.5). During the elution process, 2-ml fractions were collected. The first 4 ml of eluate contained no amylase activity whereas the next 6 ml contained 74% of the total units applied to the column. The course of purification of the enzyme is summarized in Table I. An over-all purification of approx. 3400-fold has been achieved; the final specific activity of 1670 mg maltose per mg protein per 10 min, or 488 μmoles maltose per mg protein per min, is of the

TABLE I

PURIFICATION OF α-AMYLASE FROM PEA COTYLEDONS

Enzyme activities were determined as described in the text, using soluble starch as substrate.

	Fraction No.	*Protein concn. (mg/ml)*	*Specific activity**	*Total units*	*Recovery (%)*
I	Cotyledon homogenate	8.0	0.49	2038	100
II	Heat treatment (70°)	1.9	1.66	1577	77
III	Purification with glycogen**	0.25	206	710	35
IV	Calcium phosphate–cellulose eluate	0.01	1670	527	26

* Expressed as mg of maltose liberated per mg protein per 10 min.
** Carried out with an aliquot of lyophilized Fraction II.

same order of magnitude as the specific activity values reported for pure α-amylases isolated from other sources[19].

Determination of the Michaelis constant (K_m)

The apparent Michaelis constant for the amylase was determined by use of a DIXON-WEBB[20] plot in which S/v is plotted *vs.* S. Soluble potato starch served as substrate; the enzyme preparation used had a specific activity of 143. Initial velocity (v) determinations at each substrate concentration were made by adding 0.1 ml (10 μg protein) of enzyme solution to a series of tubes containing a given concentration of soluble starch (S) in 0.1 M acetate buffer (pH 5.8). The final incubation volume was 2.0 ml. For each series of incubation tubes, the reaction was stopped at varying fixed intervals of time to ensure that true initial velocities were measured. When the substrate concentration was varied from $7.5 \cdot 10^{-3}$ to $5.0 \cdot 10^{-4}$ g/ml, the K_m value for the amylase was found to be approx. $2.0 \cdot 10^{-4}$ g/ml. The K_m values determined for α-amylases purified from several other sources are very similar in magnitude[21].

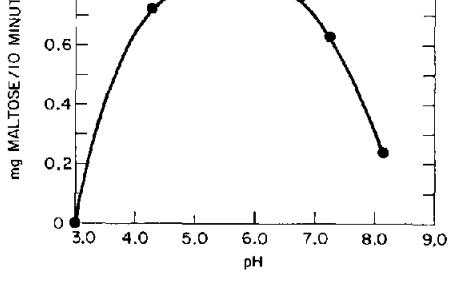

Fig. 1. Activity of pea cotyledon amylase as a function of pH. Amylase (7 μg), with a specific activity of 114, was added to each of several tubes containing 1 ml of 1% soluble starch and 1 ml of 0.025 M acetate–0.025 M phosphate buffer. The pH of the soln. in each tube was measured with the use of a glass electrode (points plotted) before adding the enzyme. After incubating the tubes for 10 min at 25°, the enzymic reaction was stopped by adding 2 ml of 3,5-dinitrosalicylic acid reagent. All results were corrected for any reducing material found in a control tube to which the 3,5-dinitrosalicylic acid reagent was added at zero time.

pH dependency of enzyme activity

The effect of pH on enzymic activity was determined over the range of values, 3.0–8.1. As can be seen, the amylase exhibits maximal activity in the buffer system used in the pH range 5.3–5.9 (Fig. 1). These values are to be compared with 4.6 and with 4.7–5.4 obtained for the pH optima of the α-amylase of sorghum[22] and that of barley malt[23], respectively.

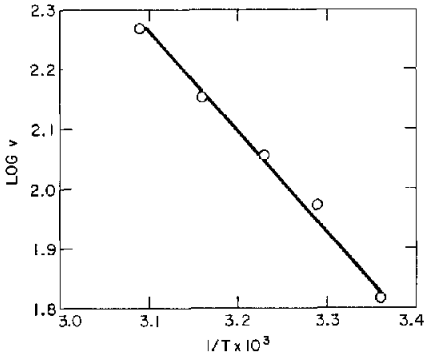

Fig. 2. Effect of temp. on the reaction catalyzed by pea cotyledon amylase. The enzyme solution was made by dissolving 200 mg of lyophilized powder (Fraction No. II, Table I) in 4 ml of 0.1 M acetate buffer (pH 5.8). Small portions of this solution (0.05 ml or less) were added to 1 ml of a 1% soluble starch solution. The final volume of the reaction mixture was brought up to 2 ml in each case by adding 0.005 M $CaCl_2$ solution. Standard assays were performed at the temperatures shown; zero-time controls were uniformly included.

Determination of activation energy (E_a)

To determine the activation energy of the reaction catalyzed by the amylase, an Arrhenius plot was made covering the temperature range, 25°–51° (Fig. 2). The value of E_a obtained from the slope of the line is 7600 cal/mole. The corresponding value found for the α-amylase purified from barley malt is 7050 cal/mole[23]. α-Amylases purified from mammalian tissue appear to have somewhat higher energies of activation[19].

Proof that the amylase is of the α-type

Studies were undertaken to determine whether the amylase of pea cotyledons is of the α- or the β-type[24]. If the enzyme were an α-amylase, as was suspected because of its stability to heating at 70° in the presence of Ca^{2+} (Table I) (ref. 24), it should possess several characteristic properties. For example, soluble starch should be hydrolyzed by the enzyme to an extent where color with iodine is no longer observed; also, a variety of oligosaccharides should be liberated as hydrolysis products; furthermore, the reducing products liberated by the enzyme-catalyzed reaction should be of the α-configuration[24].

(1) Table II shows the results obtained when formation of the starch iodine color complex is used to examine the nature of starch hydrolysis catalyzed by various amylase preparations. As can be seen, when known α-amylase of bacterial origin and the pea cotyledon amylase are separately incubated under identical conditions with soluble starch as substrate, both enzymes digest the starch with complete disad-

pearance of the starch–iodine color. In contrast, a constant residual level of color always remains when starch is incubated for prolonged periods of time with known β-amylase of malt. This residual level of color seen with incubation mixtures containing β-amylase is due to the presence of β-limit dextrins.

(2) Other investigators have shown that a number of α-amylases require low levels of calcium ion for activity; these same enzymes are also more stable in the

TABLE II

STARCH DIGESTION BY AMYLASES FROM DIFFERENT BIOLOGICAL SOURCES

Enzyme activity was followed by the disappearance of the starch–iodine color complex. The incubation mixtures contained 2.0 ml of a 1% soluble starch solution in 0.1 M acetate buffer (pH 5.8) and the following amounts of each amylase preparation: commercial bacterial α-amylase, 150 μg; commercial β-amylase of malt, 300 μg; pea cotyledon enzyme (specific activity = 232), 16 μg protein. The final volume was brought to 3.0 ml with 0.1 M acetate buffer (pH 5.8). While the reaction mixtures were incubated at 25°, 0.1 ml aliquots were removed at the times indicated and added to 5 ml of an iodine solution (0.25 M KI + 0.0035 M I_2 (ref. 25)). The resulting solution was diluted to 15 ml with H_2O and read against a reagent blank with a No. 54 filter in the Klett–Summerson photoelectric colorimeter.

Time (min)	*Per cent initial starch–iodine color*			
	*α-Amylase**	*β-Amylase***	*Pea cotyledon amylase*	*No enzyme*
0	100	100	100	100
10	44	66	77	—
20	11	56	47	99
30	4	47	22	99
40***	3	48	14	99
55	1	37	4	104
140	0	40	0	—

* Commercial α-amylase of bacterial origin.
** Commercial β-amylase obtained from malt.
*** An additional 300 μg of pea cotyledon enzyme were added to the respective incubation mixture at this time.

presence of this ion[26]. Both of these properties characterize the amylase studied here. Essentially all enzymic activity is lost by dialyzing the enzyme solution against EDTA (see section on *Inhibition studies*). When, however, calcium ions are added to pea cotyledon extracts, almost all the amylase activity remains after heating for 15 min at 70° (see section on *Purification of the amylase, Step 2*). β-Amylases are known to be denatured by such heat treatment.

(3) α-Amylases catalyze the degradation of starch in such a way that a large number of reducing substances, including glucose, maltose, maltotriose and higher oligosaccharides, are liberated. Paper chromatographic examination of the products formed when the amylase of pea cotyledons is incubated with starch shows the presence of a wide variety of reducing compounds (see section on *Action pattern studies*). When tested under identical conditions and by the same techniques, known malt β-amylase liberates only maltose.

(4) A final criterion for classifying an amylase as to type (α or β) is to determine the configuration of the reducing products released by action of the enzyme on

starch. ROBYT AND FRENCH[27] have noted that this is possibly the only valid criterion for classifying amylases into α- or β-types since they found that the amylase from *Bacillus polymyxa* exhibits properties of both types when examined by other criteria. In this test, one determines whether the products initially formed by amylase activity are of the α- or β-form. At the pH value of the enzymic digestion mixture, mutarotation occurs very slowly; the addition of alkali (sodium carbonate), however, rapidly catalyzes this process. When, therefore, alkali is added to a digest containing products of the α-configuration, as obtained from an α-amylase, a decrease in optical rotation value is observed. β-Amylases, on the other hand, release sugars having the β-configuration at the anomeric carbon atom. Accordingly, the optical rotation value of a β-amylase digestion mixture increases in magnitude. The results obtained with preparations of known α-amylase and β-amylase as well as with the pea cotyledon amylase (Fig. 3) show that the latter enzyme liberates reducing oligosaccharides which have the α-configuration at the anomeric carbon atom.

Collectively, these results establish that the enzyme purified from pea cotyledon extracts is of the α-type.

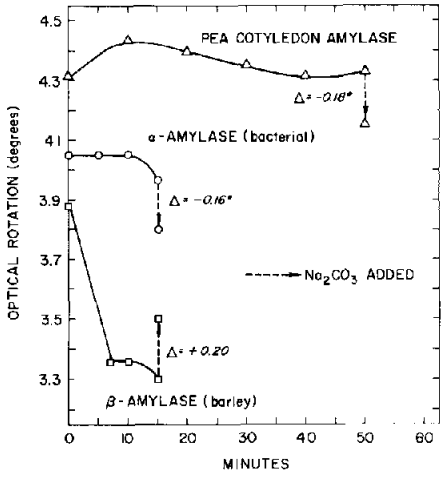

Fig. 3. Optical rotation study of the action of various amylases. A polarimeter tube (2.2 dm, 18 ml capacity) was filled with a 1% solution of soluble starch (see METHODS). The designated amylase was added in the following amounts: pea cotyledon enzyme, 0.190 mg (specific activity = 218); commercial α-amylase (from bacteria), 5.0 mg; commercial β-amylase (from malt), 5.0 mg. After the polarimeter readings reached a constant value, 10 mg of sodium carbonate were added and the direction and magnitude of change in optical rotation observed (shown by arrows).

Action pattern studies

The amylase from pea cotyledons was further characterized by examining the pattern by which it degrades several carbohydrate substrates. With amylose or amylopectin as substrate, the enzyme catalyzes the liberation of a large variety of reducing oligosaccharides. Only faint traces of low-molecular weight oligosaccharides are detected before the achroic point is reached when amylose (0.1% solution) is digested by the enzyme (Fig. 4). Oligosaccharides larger than maltohexaose appar-

ently are formed during these early stages of digestion. Beyond the achroic point, individual oligosaccharides containing six or less glucose units increase in amount with time of incubation and remain in the digestion mixture long after the achroic point has been reached. Separate incubation of the enzyme with maltohexaose (G_6), maltopentaose (G_5), maltotetraose (G_4), and maltotriose (G_3) as substrate showed these compounds are cleaved very slowly, if at all. Only significant hydrolysis of maltohexaose to either a G_4 plus a G_2 unit or to two G_3 units could be detected. Essentially

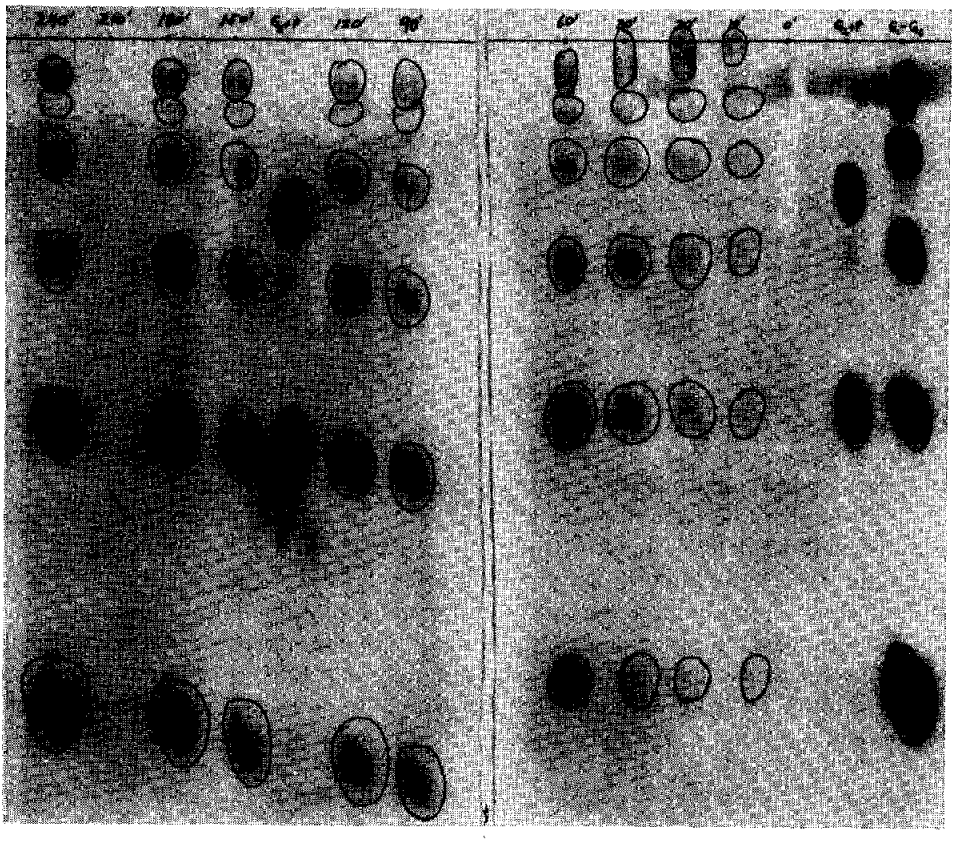

Fig. 4. Chromatographic analysis of the action pattern of pea cotyledon amylase. A saturated amylose solution (1.0 ml) was incubated with 0.1 ml of the amylase at 37° (0.1 ml of the enzyme preparation liberated 0.8 mg of maltose in 10 min in the standard assay procedure). At the times shown, 25-μl samples were removed from the incubation mixture and spotted on Whatman filter paper. The chromatogram was developed for 30 h. The achroic point of the digestion mixture was reached at 15 min. P = panose, G_1 = glucose, G_2 = maltose, G_3 = maltotriose, etc.

identical results were obtained with amylopectin (0.5% solution) as substrate. The pea cotyledon enzyme, therefore, is quite similar to the α-amylases from sorghum[28] and from malt[29] in that no significant amounts of oligosaccharide-units G_6 through G_2 appear in the digestion mixture until the achroic point is passed. The amylase reported here is also similar to other known α-amylases in that it does not catalyze any detectable degradation of cycloheptaamylose.

Inhibition studies

A general property of α-amylases is their content of calcium ion[26]. As a consequence, calcium-ion binding agents are good inactivators of enzymes of this type. The α-amylase purified from pea cotyledon extracts provides no exception to this general rule since it is readily inactivated by EDTA. When 4 μg of pea cotyledon amylase (specific activity = 244) are incubated with 0.005 M EDTA for 20 min at 25°, no activity can be detected by the routine assay procedure. Under the same conditions, 0.005 M citrate and 0.005 M oxalate inhibit enzymic activity 40% and 25%, respectively. However, dialysis of the amylase from pea cotyledons against either one of these last two agents (0.005 M, final concentration) for 15 h at 4° completely blocks all enzymic activity.

In several experiments carried out to test this point, we were not able to reverse the inactivation of the enzyme caused by EDTA. Addition of large excesses of calcium ions (final concentration four times greater than EDTA) followed by extensive dialysis against buffered calcium chloride solutions does not restore enzymic activity. The α-amylase of barley malt is readily reconstituted by similar treatment[23]. Even when the pea α-amylase is first exposed to $5 \cdot 10^{-4}$ M DFP for 24 h, for purposes of inhibiting proteases[30], and then treated with EDTA, all enzymic activity is irreversibly lost. The basis for this inability to recover amylase activity is not immediately apparent. Possibly, since this amylase has not been crystallized, proteolytic activity in the enzyme preparation is still sufficiently great to degrade the calcium-free enzyme in spite of the treatment with DFP. Also, the possibility cannot be ruled out that this particular α-amylase is not easily reconstituted once the bound calcium ion has been removed from the protein molecule.

Limited studies would seem to indicate that the amylase from pea cotyledons contains no sulfhydryl groups essential for its activity. For example, incubation of 9 μg of the partially purified enzyme (specific activity = 101) with $1 \cdot 10^{-4}$ M *p*-chloromercuribenzoate for 15 min at 25° causes no inhibition.

Apparently, the enzyme as obtained in cotyledon extracts contains sufficient calcium ion for maximal activity; no stimulation of activity is effected by adding $CaCl_2$ solution to crude extracts. Furthermore, this amylase does not appear to require chloride ion.

DISCUSSION

An amylase, present in extracts of germinated pea cotyledons, has been obtained in a high state of purity. The specific activity of the most highly-purified preparations of this enzyme is of the same magnitude as that reported for a number of amylases obtained in crystalline form. In fact, the barley malt α-amylase was crystallized after being purified only 65-fold[31], whereas a purification of at least 3400-fold is reported here for the amylase from pea cotyledons. This observation serves to indicate that the enzyme from cotyledon extracts represents only a very small fraction of the total protein in this plant storage organ. Whether or not the pea amylase is homogeneous at this stage of purification and can be obtained in crystalline form remains to be determined.

Several independent lines of proof show this enzyme is an amylase of the α-type. It possesses characteristics common with other plant α-amylases in terms

of the products formed and its stability to heat treatment in the presence of calcium ions. The pea amylase has an acidic pH optimum (5.3–5.9) as do most plant and bacterial amylases, including those from barley malt (4.7–5.4) (ref. 23), *Aspergillus oryzae* (5.5–5.9) (ref. 32), *Bacillus subtilis* (5.3–6.4) (ref. 33), sorghum (4.6) (ref. 22), and *Bacterium stearothermophilus* (4.6–5.1) (ref. 21). In contrast, amylases from mammalian tissues generally have higher pH optima, *e.g.*, 6.9 for hog pancreatic[34] and human salivary[35] amylases. The α-amylase from pea cotyledons is inactive below a pH value of 3, a property quite characteristic of amylases of this type and which provides a ready means for preferentially inactivating α-amylases in mixtures of α- and β-amylases[36]. The energy of activation for the reaction catalyzed by the enzyme from peas is 7600 cal/mole. This value is very comparable to that of 7050 cal/mole obtained by FISCHER AND HASELBACH[23] for the barley malt α-amylase.

The pea enzyme is similar to the α-amylases present in sorghum[28] and in malt[29] with regard to the pattern of attack and breakdown of amylaceous molecules. For all of these α-amylases, significant amounts of low-molecular-weight oligosaccharides (G_2–G_6) are not formed until the achroic point is reached. Once these products are liberated, they persist in the enzymic digest for at least several hours. In contrast to these plant enzymes, the α-amylase in human saliva catalyzes the release of a considerable amount of small oligosaccharides (G_2–G_4) before the achroic point[28]. The same is true for the enzyme of *B. subtilis* where, before attainment of the achroic point, oligosaccharides greater than maltoheptaose (G_7) are digested[37].

The most obvious role for the α-amylase of pea cotyledons is to catalyze the hydrolysis of starch reserves in that organ. Other findings* tend to confirm this suggestion since a rapid decrease in starch content is seen in cotyledon preparations at the time of germination when α-amylase activity is increasing rapidly. Since α-amylases catalyze a random hydrolytic breakdown of starch, a rapid degradation of the polysaccharide molecule is accomplished by this type of enzyme thereby making smaller molecular weight intermediates readily available to the developing plant. The part this enzyme plays in the germinating cotyledon and its interrelationship with other carbohydrases present in the developing seedling are considered in greater detail in the following paper.

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